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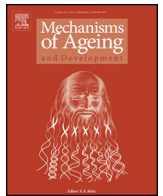
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Life-long endurance exercise in humans: Circulating levels of inflammatory markers and leg muscle size

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ABSTRACT

Human aging is associated with a loss of skeletal muscle and an increase in circulating inflammatory markers. It is unknown whether endurance training (Tr) can prevent these changes. Therefore we studied 15 old trained (O-Tr) healthy males and, for comparison, 12 old untrained (O-Un), 10 Young-Tr (Y-Tr) and 12 Young-Un (Y-Un). Quadriceps size, VO₂ peak, CRP, IL-6, TNF- α and its receptors, suPAR, lipid profile, leucocytes and glucose homeostasis were measured. Tr was associated with an improved insulin profile ($p < 0.05$), and lower leucocyte ($p < 0.05$) and triglyceride levels ($p < 0.05$), independent of age. Aging was associated with poorer glucose control ($p < 0.05$), independent of training. The age-related changes in waist circumference, VO₂ peak, cholesterol, LDL, leg muscle size, CRP and IL-6 were counteracted by physical activity ($p < 0.05$). A significant increase in suPAR with age was observed ($p < 0.05$). Most importantly, life-long endurance exercise was associated with a lower level of the inflammatory markers CRP and IL-6 ($p < 0.05$), and with a greater thigh muscle area ($p < 0.05$), compared to age-matched untrained counterparts. These findings in a limited group of individuals suggest that regular physical endurance activity may play a role in reducing some markers of systemic inflammation, even within the normal range, and in maintaining muscle mass with aging.

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1. Introduction

Aging of the human body is associated with a gradual loss of muscle mass (sarcopenia). In some cases, a critically low muscle mass, together with reduced muscle function, will result in frailty, which is a powerful predictor of mortality (Fried et al., 2001). Part of the loss of muscle mass is clearly associated with reduced levels

of physical activity and muscle disuse. However, individuals who maintain a high level of physical activity throughout life do experience some loss of skeletal muscle mass, indicating that exercise cannot completely prevent the age-related decline in muscle mass (Faulkner et al., 2008). The explanation for this remains unknown, but factors like neuro-muscular impairment, cell membrane changes or altered intracellular signaling have been demonstrated to play a role in animal models (Andersson et al., 2011; Burks et al., 2011; Narici and Maffulli, 2010). However, a comprehensive explanation for sarcopenia in humans is still lacking. A decline in adaptive immunity, or the development of low-grade inflammation with elevated levels of inflammatory markers, possibly derived from adipose tissue, may play a role (Lutz and Quinn, 2012). With regard to systemic inflammation, elevated circulating levels of the acute phase protein C-reactive protein (CRP) and cytokines, such as tumor necrosis factor alpha (TNF α) and interleukin 6 (IL-6), have been found in elderly individuals (Bruunsgaard et al., 2000) and these factors have been reported to be related to mortality (Harris et al., 1999). Similarly, the soluble urokinase plasminogen activator receptor (suPAR),

Abbreviations: CRP, C-reactive protein; HDL, high density lipoprotein; HOMA-IR, homeostatic model assessment – insulin resistance; IL-6, interleukin 6; IPAQ, International Physical Activity Questionnaire; LDL, low density lipoprotein; MET, metabolic equivalent of task; MRI, magnetic resonance image; OGTT, oral glucose tolerance test; Q-CSA, quadriceps cross sectional area; QUICKI, quantitative insulin sensitivity check index; sTNFR, soluble TNF receptor; suPAR, soluble urokinase plasminogen activator receptor; Tr, trained; TNF- α , tumor necrosis factor alpha; Un, untrained; VO₂ peak, peak oxygen consumption.

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which is also a marker of systemic low-grade inflammation, has been found to be associated with age, chronic diseases, cancer and mortality (Eugen-Olsen et al., 2010; Langkilde et al., 2011).

Inflammation is considered to play a role in disease development and prognosis (Toth et al., 2006; Visser et al., 2002), and high levels of inflammatory markers are associated with an increased risk of development of cardiovascular disease and cancer (Allin et al., 2010; Wensley et al., 2011). In addition, obesity and visceral adiposity are positively associated with elevated cytokine levels (TNF α and IL-6) and CRP (Park et al., 2005). A reduction in trunk fat induced by 10 months of cardiovascular exercise training was significantly related to a drop in circulating CRP levels (Vieira et al., 2009). SuPAR in the general population is not uniformly related to obesity and visceral adiposity but is associated with an increased risk of cardiovascular disease (Lyngbaek et al., 2013a).

Several large cohort studies have found a relationship between self-reported physical activity levels and systemic markers of inflammation: higher levels of physical activity are coupled to lower levels of circulating inflammatory markers in elderly individuals (Taaffe et al., 2000; Geffken et al., 2001; Wannamethee et al., 2002; Reuben et al., 2003; Colbert et al., 2004; Albert et al., 2004; Rahimi et al., 2005; Yu et al., 2009; Valentine et al., 2009; Elosua et al., 2005). This coupling was also evident after adjustment for other risk factors and persisted even with relatively low levels of physical activity (Elosua et al., 2005). Aerobic fitness was inversely associated with inflammatory biomarkers in persons above 60 years (Taaffe et al., 2000; Valentine et al., 2009; Rahimi et al., 2005). Intervention studies have shown that physical training over several months reduces circulating levels of CRP and IL-6 in some (Kohut et al., 2006; Vieira et al., 2009; Campbell et al., 2009) but not all studies (Nicklas et al., 2005, 2008; Hammett et al., 2004; Lund et al., 2011). It is important to note that these intervention studies were performed in patient groups or in persons that had several risk factors, and thus all had relatively high levels of inflammatory markers to begin with. Most of the studies demonstrated that training induced a reduction in chronic inflammation and weight loss, but one study (Nicklas et al., 2008) showed that regular aerobic exercise training was efficient in lowering IL-6 levels even without weight loss.

The aim of the present study was to investigate the influence of life-long endurance exercise on the levels of inflammatory markers and muscle size in healthy, normal-weight young and elderly males. We used endurance trained individuals (long-distance runners) as the active group of subjects, either young endurance athletes or elderly master athletes in order to obtain data from individuals who were healthy and had performed regular training for most of their life. As healthy control individuals we used untrained, weight-matched males for the respective age groups.

We hypothesized that age and life-long endurance training would affect systemic inflammatory levels and muscle size, and that small differences in circulatory inflammatory markers would be inversely related to muscle size. Inflammatory biomarkers were determined from circulating blood, and muscle size and function were measured. In addition, blood lipid profile, glucose homeostasis and body composition were determined to evaluate any potential coupling of these parameters to aging and endurance training.

2. Methods

2.1. Subjects

We recruited a total of 49 males: 15 male endurance runners (master athletes, old trained, O-Tr; running distance of 49 ± 3 km/wk (4–6 training days/wk) over the last 28 ± 2 years (mean \pm SE)), 12 old untrained weight matched healthy controls (O-Un), and 10 young males matched for current running distance to the old trained (young trained, Y-Tr; 43 ± 5 km/wk (4–6 training days/wk), endurance runners during the last 6 ± 1 years) and 12 young untrained weight matched controls (Y-Un). The

untrained subjects had been sedentary for at least five years and they currently did not perform any regular physical activity. The trained individuals were all endurance runners. Moreover, neither the trained or the untrained had any physically demanding occupational jobs, thus their physical activity level was primarily governed by their leisure time activities. All subjects were healthy normotensive ($<140/90$ mmHg) and non-obese (BMI <28), did not take any prescription medication, had no overt signs or symptoms of diabetes, pre-diabetes or atherosclerosis, and had no known joint, muscle or tendon pathology. The O-Un group was selected to represent a healthy age matched but untrained group to separate out the exercise component as much as possible during the aging process. Before the test days, subjects abstained from caffeine and fasted overnight to be studied in the post-absorptive state. Subjects were studied at least 24 h after their last running session to avoid the acute effects of exercise but they were still considered to be in their habitual training state. The ethics committee of the Capital Region of Denmark approved this study (journal number 25543), and all procedures conformed to the Declaration of Helsinki. Written, informed consent was obtained from all subjects before the study.

2.2. Study design

On the first test day, subjects arrived fasted in the morning and blood samples were obtained. An oral glucose tolerance test (OGTT) was performed, anthropometrics were measured and questionnaires filled in. Finally a magnetic resonance image (MRI) scan of the thigh was performed to assess the cross-sectional area (CSA) of the quadriceps muscle. On the second test day, subjects arrived, again overnight-fasted. Muscle strength (maximal voluntary contraction, MVC) was measured. One hour after consuming a light sandwich, fruit and water, maximal oxygen consumption was measured. Test days 1 and 2 were consecutive days.

2.3. Subject characteristics and physical activity level

Waist circumference was measured as the smallest circumference between anterior superior iliac spine and the lower ribs, to the nearest mm. Height was measured to the nearest mm and weight to the nearest 100 g, wearing light clothes and without shoes, and body mass index (BMI) was calculated.

To quantify the physical activity level of the subjects, the International Physical Activity Questionnaire (IPAQ) was used (Craig et al., 2003). The IPAQ questionnaire quantifies the physical activity level of the subjects as the average weekly MET (metabolic equivalent of task)-score, MET-minutes/week. One MET = $1 \text{ kcal kg}^{-1} \text{ h}^{-1}$ is equivalent to the resting metabolic rate (RMR) obtained during quiet sitting (Ainsworth et al., 2011). WHO (World Health Organization) has defined a weekly MET-score below 600 as low physical activity level and above 3000 as a high physical activity level. We also used a Danish questionnaire, which has been used in several large scale investigations of health and disease status among the Danish population (Sundhed & Sygelighed, SUSY, www.sst.dk), to assess the sedentary time of the subjects.

2.4. Muscle size, strength and exercise performance

The anatomical cross sectional area of the quadriceps femoris muscle (Q-CSA) was measured 20 cm proximal to the tibia plateau (mid-thigh level) by magnetic resonance imaging (MRI) (General Electric, Sigma Horizon LX 1.5 Tesla, T1 weighted SE) using a lower extremity coil. The images were obtained using the following parameters: TR/TE = 500/14 ms, FOV 18, matrix 512×512 and slice thickness = 6 mm. Subsequently, the lean muscle mass of the quadriceps muscle (subcutaneous and intermuscular non-contractile tissue were excluded in the measurement) was manually outlined (see Fig. 1A) using a DICOM file viewer and associated measurement software Osirix 2.7.5 (OsiriX medical imaging software, Geneva, Switzerland). The same experienced investigator completed the analysis twice (blinded to the group of the subject) and the average of these two measurements was used.

Strength of the quadriceps muscle was determined as the maximum knee extensor moment during a 10 sec ramp contraction. Subjects performed a 5-min warm-up on a stationary bike. Thereafter the subjects were seated in a custom made rigid chair with both hips and knees flexed to an angle of 90° . A leg cuff, which was connected to a strain gauge (Bofors KRG-4, Bofors, Sweden) through a rigid steel rod perpendicular to the lower leg, was mounted on the leg just above the medial malleolus. Subjects performed 4–5 isometric knee extension ramps by applying gradually increasing force until maximum. Each ramp contraction was separated by a one-minute rest period. All measurements were performed by one leg (non-dominant). During the contractions, force was sampled at 1500 Hz (Noraxon Inc.). Tibia moment was measured (from the point of fixation to the lateral epicondyle of the knee) to calculate the knee extensor moment.

Peak oxygen consumption was measured on a Monark electronically braked ergometer bike (Monark 839 Ergonomic, Proterapi, Brøndby, Denmark) using an incremental protocol with a 25 W increase in load every minute, starting at 50 W (O-Un) or 75 W (all other groups) until volitional exhaustion, which was reached between 4 and 12 min for all subjects. Subjects were instructed to aim at a pedalling frequency of approximately 80–90 revolutions per minute (RPM), and were verbally encouraged to perform their max. Oxygen consumption (VO_2), CO_2 excretion, respiratory exchange ratio (RER), heart rate (HR), minute ventilation (VE) and

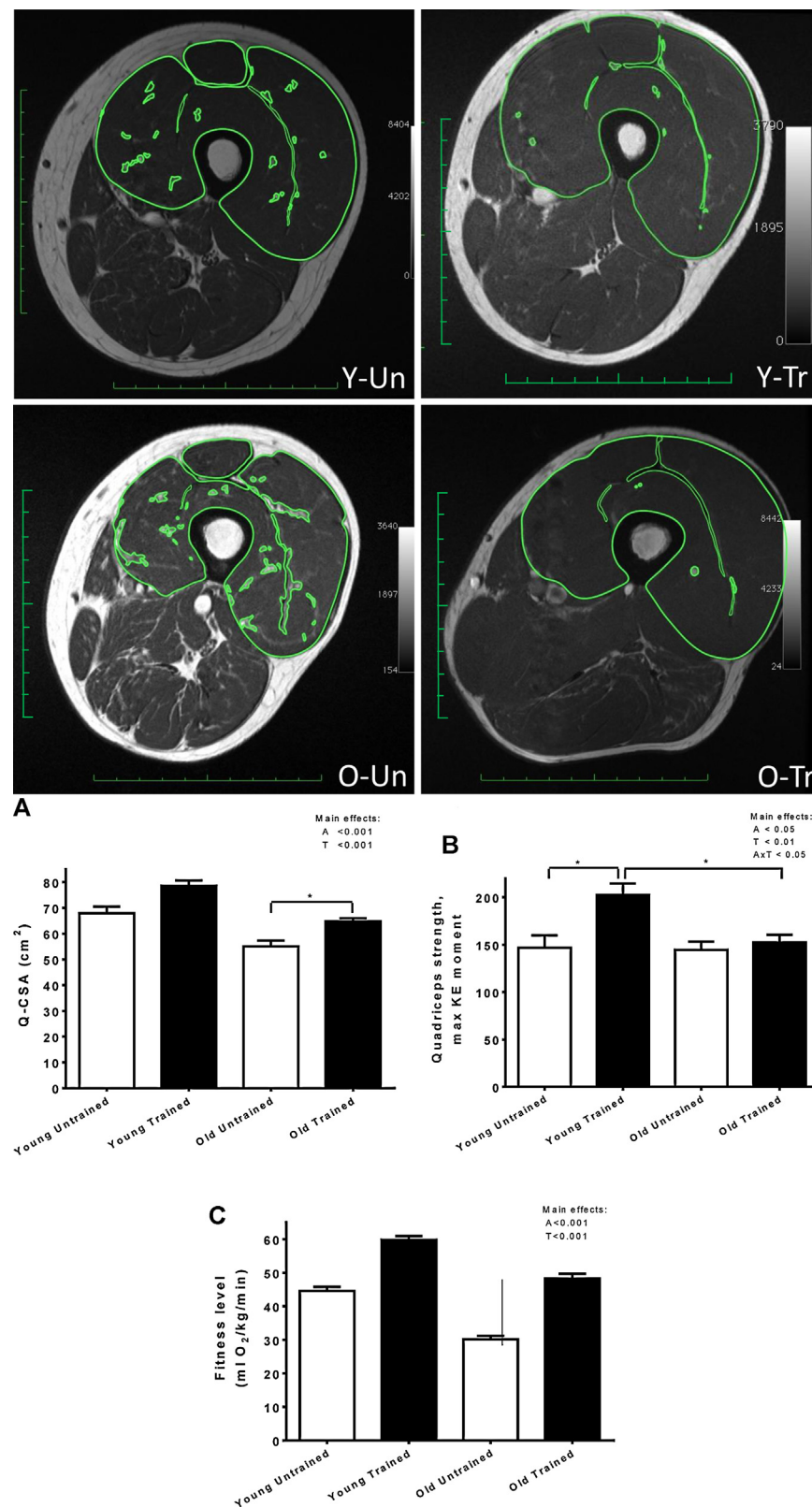


Fig. 1. Muscle size, strength and exercise performance. (A) Quadriceps cross-sectional area (Q-CSA), (B) quadriceps muscle strength and (C) peak oxygen uptake in the four different subject groups. Data are shown as mean + SE. (A) Upper panel: Magnetic resonance image (MRI) of the quadriceps muscle of young (top row; Y-Un (left) and Y-Tr (right)) and old (bottom row; O-Un (left) and O-Tr (right)) subjects used to quantify Q-CSA. The images were obtained at mid-thigh level, 20 cm proximal to the tibia plateau. The lean muscle mass of the quadriceps muscle was manually outlined as shown by the drawn lines, excluding subcutaneous and intermuscular non-contractile tissue. Scale bar = 10 cm, 1 cm per indentation. Lower panel: Quantification of Q-CSA as measured by MRI. Q-CSA was significantly influenced by age (A, $p < 0.001$) and training (T, $p < 0.001$), p -values for these main effects are given in figure. $N = 12$ in Y-Un, 10 in Y-Tr, 11 in O-Un and 12 in O-Tr. * $p < 0.001$ for difference between O-Un and O-Tr. (B) Quadriceps muscle strength was determined as maximum knee extensor (KE) moment during a 10 s ramp contraction and significant main effects of age (A, $p < 0.05$) and training (T, $p < 0.01$) were observed, as well as a significant interaction (A×T, $p < 0.05$) with Y-Tr being significantly stronger than O-Tr and Y-Un, * $p < 0.05$. $N = 10$ –15 as given in Table 1. (C) Peak oxygen consumption (VO_2 peak) was determined as the highest value reached during a 15 s period, and shown as fitness level (ml O₂/kg/min). Significant effects of age (A) and training (T) were observed, p -values are given in figure. $N = 10$ –15 as given in Table 1.

cadence (RPM) were continuously recorded (breath-by-breath) using Masterscreen CPX equipment (Care Fusion, San Diego, CA). All subjects reached RER values above 1.09 (range 1.09–1.34), indicating that high exertion levels were obtained. Peak oxygen uptake (VO_2 peak) was determined as the highest value reached during a 15 s period (ignoring the highest and lowest value within that interval), and given as absolute values ($\text{ml O}_2/\text{min}$) and fitness level ($\text{ml O}_2/\text{kg}/\text{min}$).

2.5. Blood samples

Fasted blood samples were drawn from an antecubital vein. For analyses of insulin, CRP, IL-6, TNF α and its soluble receptors and suPAR, whole blood was drawn into EDTA tubes and cooled on ice for 10 min, followed by centrifugation (10 min at $3060 \times g$ at 4°C), and the plasma phase was stored at -80°C until analysis.

For analyses of sTNFRI and sTNFRII, whole blood was drawn into clot activator tubes and allowed to clot at room temperature for 30 min followed by centrifugation (10 min at $3060 \times g$ at 4°C), and the serum phase was stored at -80°C until analysis.

2.5.1. Blood lipid profile

Blood lipid profile (triglycerides, total cholesterol, high-density lipoprotein (HDL) and low-density-lipoprotein (LDL) cholesterol) was determined at the Clinical Biochemistry Department, Bispebjerg Hospital, Copenhagen. These blood samples were collected into vacutainers and analysed no more than 2 h after the time of collection, according to the laboratory standard operating procedures (Nordin et al., 2004). Authorized lab-technicians handled the analyses. Cholesterol, LDL, HDL, and triglyceride concentrations were measured by a colorimetric slide test in a Vitros 5.1 FS (Ortho Clinical Diagnostics, Raritan, New Jersey, USA) (Sennels et al., 2011, 2012).

2.5.2. Glucose homeostasis

A glucose tolerance test was performed by oral administration of 75 g of glucose dissolved in water. Glucose and insulin were analysed in blood samples obtained before glucose ingestion, and glucose levels were determined after 2 h. Blood glucose concentration was measured by use of a colorimetric slide test on Vitros 5.1 FS (Ortho Clinical Diagnostics, Raritan, New Jersey, USA). HbA1c was measured using an ion-exchange high-performance liquid chromatography method (Osoh G8-Tosoh Bioscience, Yamaguchi, Japan) (Dunn et al., 1979; Mosca et al., 1986). Both blood glucose concentration and HbA1c were determined at the Clinical Biochemistry Department, Bispebjerg Hospital, Copenhagen, where lab-technicians handled the analyses.

Plasma insulin concentration was measured using a standard insulin ELISA kit, K6219 (Dako Denmark A/S, Glostrup, Denmark). The homeostatic model assessment (HOMA) used to quantify insulin resistance (HOMA-IR) was calculated from fasting glucose and insulin as follows: $\text{HOMA-IR} = (\text{glucose in mmol/l} \times \text{insulin in mU/l})/22.5$. The quantitative insulin sensitivity check index (QUICKI) was derived from fasting insulin and fasting glucose as follows: $\text{QUICKI} = 1/(\log(\text{insulin } \mu\text{U/ml}) + \log(\text{glucose mg/dl}))$.

2.5.3. Systemic inflammatory markers

Leucocyte concentrations were measured at the Clinical Biochemistry Department, Bispebjerg Hospital, by optical spread light in a Sysmex XE (Sysmex, Kobe, Japan) (Sennels et al., 2011).

For analyses of plasma C-reactive protein (CRP), an ELISA was developed with the DuoSet DY1707 (R&D Systems, Minneapolis, MN, USA) and the coefficient of variation (CV) was: intra-plate 1.1–3.1%, inter-plate 7–10%. The following ELISA kits were used for measuring IL-6, TNF α and its soluble receptors, sTNFRI and sTNFRII: IL-6 (Human IL-6 Quantikine HS ELISA Kit HS600B), TNF α (HSTA00D), sTNFRI (DRT100) and sTNFRII (DRT200), all R&D systems. TNF α , IL-6 and CRP were measured in plasma and sTNFRI & sTNFRII in serum.

The soluble urokinase-type plasminogen activator receptor (suPAR) was measured in plasma using a commercially available sandwich ELISA-kit (ViroGates A/S, Birkerød, Denmark), according to the manufacturer's instructions. All samples were measured in duplicate, and the mean suPAR concentration of the two measurements was used for analysis. The variance between the two measurements was within the acceptable variance of 10% (range 0.0–7.8%).

2.6. Statistics

Effects of age (A), training (T) and age \times training interaction (A \times T) were tested using two-way ANOVA, with Holm–Sidak multiple comparison post hoc testing, unless otherwise stated. When a significant interaction was observed, the post hoc test was used to examine effects of training within the old (O-Tr vs O-Un), training within the young (Y-Tr vs Y-Un), age within the trained (O-Tr vs Y-Tr) and age within the untrained (O-Un vs Y-Un). Since the question whether life-long endurance training could preserve muscle mass was central to the study, the difference in Q-CSA between O-Un and O-Tr was tested using an unpaired *t*-test. Blood protein data (insulin, CRP, IL-6, suPAR, TNF α and sTNFRI & II) were log-transformed before statistical analyses. Age, IPAQ and sedentary time data were analysed using the non-parametric one-way ANOVA (Kruskal–Wallis) test, with Dunn's multiple comparison post hoc test to compare selected groups, as listed above. Height, weight, BMI and waist circumference were analysed using an ordinary one-way ANOVA with Sidaks multiple comparison post hoc test to compare selected groups, as listed above. Data are given as mean (SD) or mean (SE), as stated in the results section. SigmaPlot version 12.4 (Systat Software Inc) and Graph Pad Prism version 6 (GraphPad Software Inc, La Jolla, CA, USA) were used for statistical analyses. The level of statistical significance is given as either $p < 0.05$, $p < 0.01$ or $p < 0.001$.

3. Results

3.1. Subject characteristics (Table 1)

Anthropometric data for the subjects revealed 4 groups of normal weight, non-obese individuals. BMI was slightly higher in O-Un than in O-Tr (Table 1). Waist circumference was higher in (O-Un) than both O-Tr (*) and Y-Un (§) (Table 1).

3.2. Physical activity (Table 1)

Habitual running distance per week (mileage in km) was similar in the O-Tr ($49 \pm 3 \text{ km/wk}$) and Y-Tr ($43 \pm 5 \text{ km/wk}$ NS) groups (Table 1). Likewise, running distance during the last week prior to the

Table 1
Subject characteristics and physical activity.

Variable	Young untrained Y-Un	Young trained Y-Tr	Old untrained O-Un	Old trained O-Tr	<i>p</i> -Value
Subject characteristics	Mean (SD)				
N	12	10	12	15	
Age (years)	24 (3) [§]	26 (4) [#]	66 (4)	64 (4)	$p < 0.001$, K–W
Height (cm)	178 (6)	179 (4)	175 (4)	176 (5)	NS
Weight (kg)	70 (8)	73 (6)	75 (4)	71 (6)	NS
BMI (kg/m^2)	22 (2) [§]	23 (2)	25 (2)	23 (2)	$p < 0.05$
Waist circumference (cm)	80 (5) [§]	79 (5)	90 (4) [*]	83 (5)	$p < 0.001$
Physical activity	Mean (SE)				
Running distance/wk (km)		43 (5)		49 (3)	
Running distance last week (km)		48 (7)		43 (5)	
Running years		6 (1)		28 (2)	
IPAQ (MET-min/week)	919 (199) [†]	3724 (579)	1277 (575) [*]	8881 (1791)	$p < 0.001$, K–W
Sedentary time (min/d)	560 (34)	489 (48)	520 (61)	384 (71)	$p < 0.05$, K–W

Subject characteristics and physical activity level are shown for the four groups of subjects. *p*-Values from one-way ANOVA are given. For further details, see text. K–W: Kruskal–Wallis test.

^{*} Different from O-Tr ($p < 0.05$).

[†] Different from Y-Tr ($p < 0.05$).

[#] Different from O-Tr ($p < 0.05$).

[§] Different from O-Un ($p < 0.05$).

Table 2
Blood lipid profile and leucocytes.

Variable	Young untrained Y-Un	Young trained Y-Tr	Old untrained O-Un	Old trained O-Tr	p-Value	
					A = age T = training	Interaction age × training
Blood lipid profile	Mean (SE)					
Triglycerides (mmol/l)	1.21 (0.20)	0.73 (0.06)	1.24 (0.15)	0.70 (0.04)	T < 0.001	
Total cholesterol (mmol/l)	5.18 (0.42)	4.44 (0.19)	6.67 (0.27)	5.76 (0.24)	A < 0.001	
					T < 0.01	
HDL cholesterol (mmol/l)	1.44 (0.07)	1.49 (0.08)	1.60 (0.11)	1.93 (0.09)	A < 0.01 (T = 0.051)	
LDL cholesterol (mmol/l)	3.20 (0.39)	2.61 (0.15)	4.51 (0.22)	3.51 (0.18)	A < 0.001 T < 0.01	
Leucocytes	Mean (SE)					
Leucocytes (x10^9/L)	6.59 (0.38)	4.69 (0.36)	5.65 (0.40)	5.07 (0.24)	T < 0.001	(A×T = 0.074)

Data on blood lipid profile and leucocytes are shown for the four groups of subjects. *p*-Values for significant main effects of age (A), training (T) and interaction (A × T) are given. For further details, see text.

study was similar between these groups (O-Tr 43 ± 5; Y-Tr 48 ± 7 km/wk, NS). The O-Tr had been running for 28 ± 2 years and the Y-Tr for 6 ± 1 years (Table 1). Physical activity level assessed by IPAQ was higher in O-Tr than in O-Un (*p* < 0.001) and in Y-Tr than in Y-Un (*p* < 0.05), but similar between O-Tr vs Y-Tr and O-Un vs Y-Un (NS). A significant overall difference between the groups was found with respect to sedentary time, but none of the selected comparisons were significant.

3.3. Muscle size, strength and exercise performance (Fig. 1)

Quadriceps muscle cross sectional area (Q-CSA) was larger in the young than old (main effect of age, A, *p* < 0.001) and in the trained than the untrained (main effect of training, T, *p* < 0.001, Fig. 1A). Among the old groups O-Tr had significantly larger Q-CSA than O-Un (*p* < 0.001). Quadriceps muscle strength (Fig. 1B) revealed a significant interaction between age and training (A × T, *p* < 0.05) with Y-Tr being stronger than both Y-Un and O-Tr (*p* < 0.05). Peak oxygen consumption (ml O₂/min) and fitness level (ml O₂/kg min) (Fig. 1C) were both higher in the young compared to the old (A, *p* < 0.001) and in the trained compared to the untrained (T, *p* < 0.001) whereas no significant interaction between age and training was observed.

3.4. Blood lipid profile (Table 2)

Training was associated with a lower level of triglyceride (T, *p* < 0.001), total cholesterol and LDL cholesterol level (T, *p* < 0.01). For triglycerides no effect of aging per se was demonstrated, whereas aging was associated with a rise in total cholesterol, HDL and LDL (A, *p* < 0.01).

3.5. Glucose homeostasis (Table 3)

Fasted insulin levels were lower in the trained groups than in the untrained (T, *p* < 0.001), but unaffected by age (Table 3). Fasting glucose was lower in the young than in the old groups (A, *p* < 0.001). An interaction was observed (A × T, *p* < 0.05) with fasting glucose being lower in O-Tr vs O-Un (*) and in Y-Un than in O-Un (§). Likewise, blood glucose 2 h after glucose ingestion and glycosylated hemoglobin (HbA1c) were lower in the young than in the old groups (A, *p* < 0.01), but were unaffected by training. Insulin resistance assessed by HOMA-IR was lower in the trained than in the untrained groups (T, *p* < 0.001), and was not affected by age, and in accordance, insulin sensitivity assessed by QUICKI was higher in the trained than in the untrained groups (T, *p* < 0.001), and not affected by age (Table 3).

3.6. Systemic inflammatory markers

There was a significant effect of age and training for CRP and IL-6, and the level of inflammatory markers was lower in the young compared to the old (A, *p* < 0.01) and in the trained compared to the untrained groups (T, *p* < 0.001, Fig. 2). SuPAR was higher in the old vs young groups (A, *p* < 0.001, Fig. 2), and the interaction between age and training approached significance (A × T, *p* = 0.054). As shown in Fig. 3, an age-training interaction was present for TNFα (A × T, *p* < 0.05) and the old trained had higher TNFα than old untrained (*p* < 0.05). The level of sTNFRI was lower in the young groups compared to the old groups (A, *p* < 0.05), and in the trained groups compared to the untrained groups (T, *p* < 0.05). Likewise, serum sTNFRII was lower in the young groups compared to the old groups (A, *p* < 0.05), but no effect of training

Table 3
Glucose homeostasis.

Variable	Young untrained Y-Un	Young trained Y-Tr	Old untrained O-Un	Old trained O-Tr	p-Value	
					A = age T = training	Interaction age × training
Glucose homeostasis	Mean (SE)					
Insulin (fasted, pmol/l)	22.8 (2.8)	14.9 (1.9)	26.0 (2.4)	13.9 (1.1)	T < 0.001	
Glucose (fasted, mmol/l)	4.58(0.10) [§]	4.66 (0.11)	5.26 (0.10) [*]	4.91 (0.09)	A < 0.001	A×T < 0.05
Glucose (2 hrs, mmol/l)	4.70 (0.15)	4.49 (0.34)	5.53 (0.20)	5.05 (0.27)	A < 0.01	
HbA1c (mean mmol/l)	5.51 (0.15)	5.58 (0.16)	6.45 (0.11)	6.03 (0.16)	A < 0.001	
HOMA-IR	0.78 (0.11)	0.53 (0.09)	1.02 (0.10)	0.51 (0.04)	T < 0.001	
QUICKI	0.41 (0.01)	0.44 (0.01)	0.39 (0.01)	0.44 (0.01)	T < 0.001	

Data on glucose homeostasis are shown for the four groups of subjects. *p*-Values for significant main effects of age (A), training (T) and interaction (A × T) are given. For further details, see text.

^{*} Different from O-Tr (*p* < 0.05).

[§] Different from O-Un (*p* < 0.05).

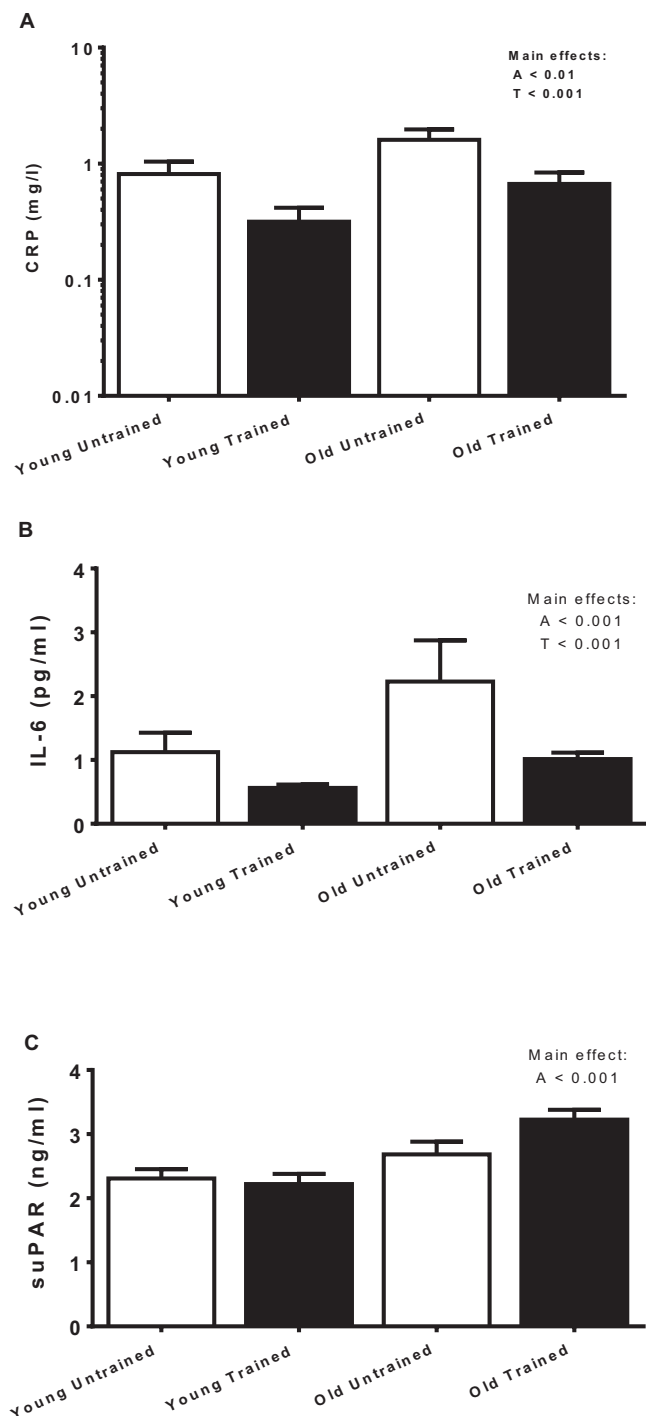


Fig. 2. CRP, IL-6 and suPAR. Level of (A) C-reactive protein (CRP), (B) interleukin-6 (IL-6) and (C) suPAR in plasma from the four different groups. All three variables were log-transformed before statistical analyses, and CRP shown on a log scale on the graph. *p*-Values from 2-way ANOVA are given in the figures. Data are shown as mean + SE. *N* = 10–15 as given in Table 1.

was observed (Fig. 3). Leucocyte levels in the endurance trained groups were significantly lower than in the untrained groups (T, $p < 0.001$), and the level was not affected by age (Table 2).

4. Discussion

To the best of our knowledge, this is the first study to investigate the interaction between the influence of aging and

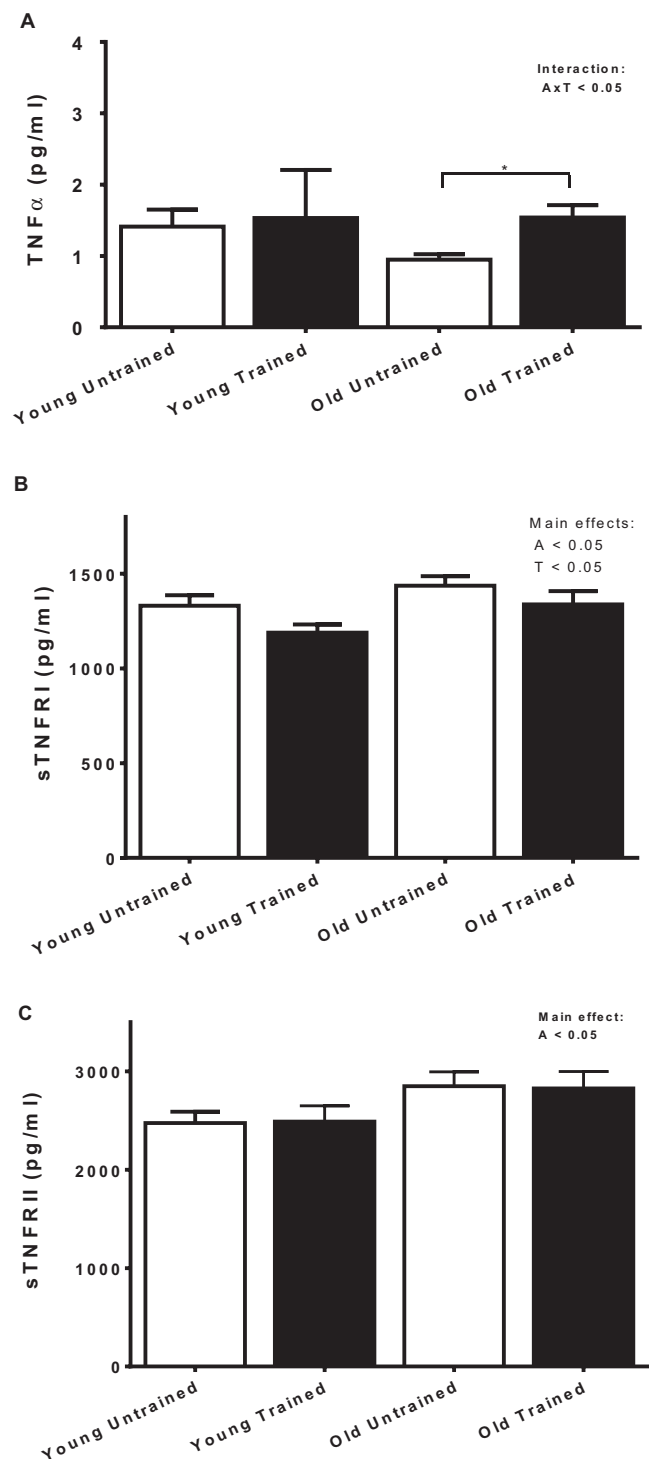


Fig. 3. TNFα and receptors. Level of (A) TNFα and the soluble receptors, (B) sTNFR I and (C) sTNFR II in the four different groups. TNFα was measured in plasma and sTNFR I & sTNFR II in serum. *p*-Values for 2-way ANOVA are given in the figures. Significant difference between O-Un and O-Tr, $*p < 0.05$. Data are shown as mean + SE. *N* = 10–15 as given in Table 1.

physical activity on inflammatory biomarkers and skeletal muscle mass in elderly master athletes. One of the major findings of the present study was that life-long endurance exercise was associated with a larger muscle size than O-Un and an attenuation of the age-related increase in inflammatory markers. Moreover, endurance training was associated with improved insulin sensitivity and lower triglyceride levels, and these effects were independent of

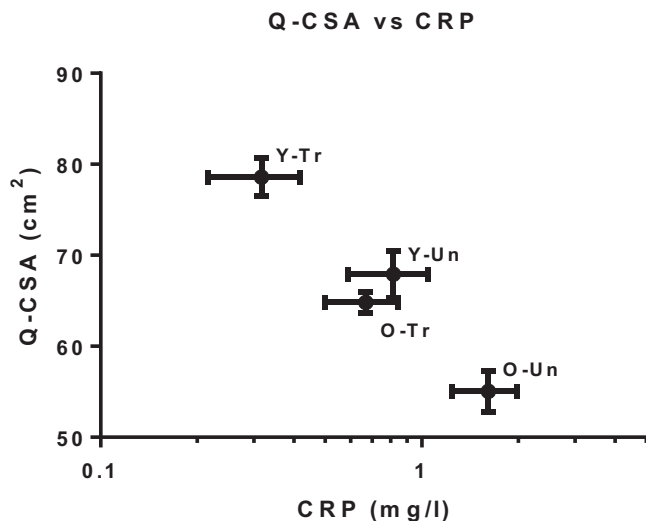


Fig. 4. Quadriceps cross sectional area (Q-CSA) and plasma CRP. Association between CRP and Q-CSA plotted as mean and SE in the four groups (Y-Tr, Young Trained $n = 10$; Y-Un, Young Untrained $n = 12$; O-Tr, Old Trained $n = 12$; O-Un, Old Untrained $n = 11$).

age. Furthermore, both age and training status were observed to influence waist circumference, VO_2 peak, total cholesterol, LDL, leg muscle size, CRP and IL-6, although age and training exerted opposing effects; aging generally affects these parameters in an unhealthy direction, whereas endurance training influences them in a health-promoting direction. Together these findings indicate that regular endurance training may play a role in lowering some markers of systemic inflammation – even within the normal range – and in regulating important metabolic and physiological muscle parameters with aging.

It has been suggested that the influence of physical training on systemic inflammation is exerted through weight loss and a loss of adipose tissue (Vieira et al., 2009). It would appear that the findings of the present study support this, in that long distance running in young and old healthy males was associated with lower levels of CRP and IL-6 (Fig. 2). However, the participants of our study had a BMI classification of normal weight, with low concentrations of CRP (approximately 1 mg/L), in contrast to the study by Vieira, where participants had a BMI classification of overweight and had higher CRP values (approximately 3 mg/L). This suggests that endurance exercise exerts a beneficial effect on systemic inflammatory status, which is unrelated to overweight. In addition, it is possible that the lower level of CRP and IL-6 in endurance trained athletes is coupled to the maintenance of skeletal muscle mass (Fig. 4). However, such a coupling is at this point speculative and needs larger studies for confirmation.

With regard to muscle mass and strength, we found a greater muscle size (Q-CSA) and muscle strength in young compared to old men, and that endurance trained individuals had greater Q-CSA and strength than their untrained counterparts (Fig. 1), which is in accordance with previous observations (Galloway et al., 2002; Tarpenning et al., 2004; Wiswell et al., 2001; Faulkner et al., 2008). Within the young groups, quadriceps muscle strength was higher in trained than in untrained individuals, which was not the case within the old groups. This would seem to suggest that muscle strength cannot be maintained by life-long endurance training. However, it should be kept in mind that all participants were selected and healthy, and thus are probably not representative of the general aging population. Furthermore, it should be noted that in spite of a training-associated difference in muscle cross sectional area in elderly individuals, as mentioned above there was no significant difference in quadriceps strength between O-Un and

O-Tr, which would indicate that the relative muscle force (per muscle area) is lower in trained vs untrained. This is very unlikely and points more towards a limitation in the strength data obtained in the present study. Variation in the measurements of strength was higher than in the CSA measurements (CV 19–31% vs 6–14% for strength and CSA, respectively) and further differences between groups in muscle strength may have emerged had a larger number of subjects been studied.

As an indicator of aerobic capacity, we observed a higher VO_2 peak in trained vs untrained, and that younger individuals had a higher VO_2 peak when compared to elderly individuals, regardless of training status, which is supported by earlier reports (Hagberg et al., 1998; Hawkins et al., 2001; Rogers et al., 1990; Wiswell et al., 2001).

In this study, both aging and physical activity levels were observed to influence blood cholesterol, LDL and to some extent HDL (Table 2). Aging was associated with elevated total cholesterol and LDL, but exercise training reduces these parameters (Table 2). These findings support the isolated findings reported earlier on aging and training (Leon and Sanchez, 2001; Vogel et al., 2009). Old athletes have previously been shown to have more favourable lipid profiles than sedentary subjects and even similar to those of young adults (Yataco et al., 1997).

While we did not determine insulin sensitivity using euglycemic, hyperinsulinaemic glucose clamp, but instead used indirect methods, fasted insulin and indexes of insulin resistance (HOMA-IR) and insulin sensitivity (QUICKI) were observed to be more favourable in the trained than untrained state, as expected (Seals et al., 1984; Henriksson, 1995; Ryan, 2000). Interestingly, there was no effect of aging on these parameters, indicating that age-related loss in insulin sensitivity is almost exclusively due to a decline in physical activity level rather than to aging per se. This supports the view that life-long physical endurance training can maintain metabolic health in human skeletal muscle. In contrast to insulin sensitivity, glucose levels (including HbA1c) were observed to be affected by age but not by training status, indicating that aging does affect glucose levels, despite the lack of influence on insulin sensitivity. It should be noted here that the O-Un individuals were screened not to have any morbidities or signs of pre-morbidity and thus do not represent the general aging population. We did this to separate out the exercise component as much as possible from the aging process.

In large scale population-based studies, relationships between inflammatory markers and loss of muscle mass and strength have previously been observed (Visser et al., 2002; Schaap et al., 2009, 2006; Geffken et al., 2001; Taaffe et al., 2000; Yu et al., 2009; Wannamethee et al., 2002; Reuben et al., 2003; Colbert et al., 2004; Valantine et al., 2009). In the majority of the cross-sectional studies, increasing time spent on physical activity was associated with a reduced level of CRP, IL-6 and in a few cases $\text{TNF}\alpha$. To our knowledge, this is one of the first studies on systemic levels of inflammatory markers in elderly athletes, including an age-matched healthy control group, and a comparison to both trained and untrained young counterparts. The positive effect of training that we observed for CRP and IL-6 was as expected based on studies showing that both of these inflammatory markers are usually lower in well trained subjects and can be reduced by long-term training (Nicklas et al., 2008; Kohut et al., 2006; Campbell et al., 2009). Furthermore, we detected greater levels of CRP and IL-6 in the old groups. Both CRP and IL-6 levels were very low in our subjects (approximately 1 mg/l for CRP), which is somewhat in contrast to earlier training studies that were mostly performed on patients with CRP levels of about 4–5 mg/l (Nicklas et al., 2008; Campbell et al., 2009). This supports the idea that, even within a range of very low CRP values, circulating levels of biomarkers are related to the degree of physical training.

The increase in suPAR with age in the present study is in line with prior studies although all suPAR concentrations in general were low. SuPAR's association with diseases related to a sedentary lifestyle, such as diabetes and cardiovascular diseases, is well established (Eugen-Olsen et al., 2010; Lyngbaek et al., 2013a), and is similar to CRP. CRP in these settings is associated with anthropometric measures, whereas suPAR is linked to endothelial dysfunction and subclinical organ damage due to atherosclerosis (Lyngbaek et al., 2013b). SuPARs are present on vascular endothelial cells and involved in several immune functions including migration, adhesion, angiogenesis, fibrinolysis and cell proliferation (Eugen-Olsen et al., 2010), making it a potential player in locally induced inflammation and regeneration. At the time of writing, only one study on circulating levels of suPAR in relation to exercise could be found, where, in well-trained young individuals, no influence of an acute bout of exercise was detected (Sanchis-Gomar et al., 2013), but it is clear that more data are needed on any potential influence of exercise on suPAR and what the implications of changed levels would be. The fact that suPAR did not demonstrate any relationship to degree of physical activity could be due to suPAR being a marker of minor pathology rather than an effect of physical training itself. In this way suPAR cannot be directly compared with the other markers of inflammation, but we cannot rule out that we, in this study due to the low number of subjects, may have overlooked any true effect of training on suPAR.

TNF α is considered a pro-inflammatory cytokine which contributes to breakdown and wasting of skeletal muscle (Li and Reid, 2001). In a 5-year study of 70-year olds, higher baseline levels of several cytokines, but in particular TNF α and its soluble receptors, were found to be associated with a greater decline in thigh muscle area, even in weight-stable individuals (Schaap et al., 2009). In the present study, the plasma levels of TNF α were generally very low and yet higher levels were detected in the old trained compared to old untrained. This was in contrast to our hypothesis, since studies in elderly individuals have shown either reduced (Phillips et al., 2012; Kohut et al., 2006; Ogawa et al., 2010; Onambele-Pearson et al., 2010; Cordova et al., 2011) or unchanged (Onambele-Pearson et al., 2010) circulating TNF α levels with training, although mainly resistance training interventions were used in these previous studies. The reduced TNF α level we observed was not a general effect of training, since it was only present in the old untrained. It is possible that an explanation for this result lies in the age and excellent health status of the old untrained in this study. Support for the latter is illustrated in the similar muscle strength measured for the young untrained and old untrained groups, indicating that the muscle of the old untrained had not "aged" much from the point of view of function. It has previously been reported that individuals in the age range 55–65 do not have elevated circulating TNF α levels compared to 18–30 year olds. However, greater levels were detected in 81-year olds and even greater levels again in centenarians, suggesting that TNF α levels do not rise with age until the ninth decade of life (Bruunsgaard et al., 1999). The soluble receptors for TNF were both slightly elevated in the old groups. An elevated circulating level of the pro-inflammatory cytokine TNF α , number of TNF α receptors and sensitivity to TNF α with increasing age has previously been found in most (Bruunsgaard et al., 2000; Mariani et al., 2006; Shurin et al., 2007; Stowe et al., 2010; Roubenoff, 2007) but not all (Donato et al., 2008) studies. In the present study, the trained groups had lower sTNFRI, whereas sTNFRII appeared unaffected by training. We measured sTNFRs together with TNF α , since TNF α is usually present at very low levels (particularly in healthy subjects) whereas sTNFRs are present in the circulation at all times and can be regarded as surrogate markers for TNF α (Stowe et al., 2010).

On average the old groups were 40 years older than the young groups, and life-long endurance training among these elderly

individuals improved a number of the measured parameters (Q-CSA, VO₂peak, CRP, IL-6 and sTNFRI) to the levels observed among the young individuals. Mostly corresponding to young untrained, or sometimes even at level of the young trained, indicating that life-long endurance trained athletes are similar to individuals 40 years younger in terms of inflammatory biomarker profile. It has however to be mentioned that the low number of individuals in the present study somewhat limits the overall conclusions that can be drawn, and larger studies are needed to confirm our findings.

Clearly, the design of the present study was cross-sectional and therefore no conclusions about cause and effect relationships between physical activity, inflammatory markers and muscle mass can be drawn. Nevertheless, the master athlete model is used as a unique model for successful, optimal aging, and serves as an example for physical activity to maintain health. Changes detected in the old trained are considered to be the result of primary (physiological) aging rather than secondary (lifestyle-related) aging (Tanaka and Seals, 2003), and thus we suggest that life-long endurance training could contribute to the changes observed in physiological parameters between the groups.

5. Conclusion

In non-obese healthy males, endurance training was associated with improved insulin sensitivity and lower triglyceride levels, which was independent of age. Furthermore, age-related changes in waist circumference, VO₂ peak, cholesterol, LDL, leg muscle size, CRP and IL-6 were counteracted by the effects of physical activity level in the old (and young) groups. Finally, life-long endurance exercise was associated with attenuation in both the age-related elevation of some inflammatory markers (CRP and IL-6) and a reduction in thigh muscle size. Overall, the findings in a limited number of individuals suggest that regular physical endurance activity may play a role both in reducing the systemic inflammation levels, even within a normal range of plasma concentration, and for maintenance of muscle mass with aging.

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